Original Article

Identification of Dermatophyte and Nondermatophyte Molds Isolated from Animal Lesions Suspected to Dermatomycoses

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Abstract

Background: Dermatomycoses contain superficial fungal infections of keratinized layers of the body such as skin, hair, and nail that affect more than 20%–25% of people and animals worldwide. Some fungi can cause superficial infections in animals after accidental penetration and colonization on injured skin and can be transmitted to humans by exposure. The infection caused mainly by dermatophyte species and may also be caused rarely by yeasts and nondermatophytic molds. **Materials and Methods:** Eighty-two skin scrapings and hair samples were collected from animals (sheep, cow, cat, camel, calf, goat, horse, and dog) in three specialized pet clinics and three livestock and slaughterhouses. The isolates were identified using direct microscopy, culture, and polymerase chain reaction-sequencing of ITS1-5.8SrDNA-ITS2 region. **Results:** Thirteen mold strains out of 82 clinical samples (15.8%) were isolated from animal lesions. *Acremonium exuviarum* (n = 4; 30.7%), *Sarocladium implicatum* (n = 2; 15.4%), *Arthroderma otae* (n = 2; 15.4%), *Chaetomium iranianum* (n = 1; 7.7%), *Trichothecium roseum* (n = 1; 7.7%), *Lichtheimia ramosa* (n = 1; 7.7%), *Penicillium chrysogenum* (n = 1; 7.7%), and *Microsporum equinum* (n = 1; 7.7%) were isolated from clinical specimens. **Conclusion:** Since opportunistic fungi are increasing as etiological agents of dermatomycoses, isolation of these molds from wounds can be a warning to veterinarians, and daily cleaning of wounds with a proper disinfectant is recommended for the prevention of fungal colonization.

Keywords: *Animal lesions, dermatomycoses, dermatophytes, opportunistic molds, zoonosis*

Introduction

Dermatomycoses contain superficial fungal infections of the skin, hair, and nail that affect more than 20%–25% of the people and animals worldwide, particularly in tropical and subtropical regions. These infections caused by yeasts, dermatophyte species, and hyaline or dematiaceous molds.[1] The frequency of infection and the distribution of causative agents can alter substantially according to geographical region, population migration profiles, climate, socioeconomic status, condition of animal husbandry, and cultural factors.[2,3] Opportunistic mycoses are uncommon, most regularly revealed as cutaneous infections in cats or as systemic hyalohyphomycosis in dogs.[4,5] The prevalence of dermatomycoses has increased considerably in animals over the past 20 years. Some fungi can cause superficial infections in animals after accidental penetration and colonization on injured skin, particularly when immunologic defects exist in the host.[6] The aim of the present study was to identify the molds isolated from animal lesions suspected to dermatomycoses.

Materials and Methods

In this cross-sectional study, 82 skin scrapings and hair samples were collected from animals in three specialized pet clinics located in Mardavij street, Northern Sheikh Sadough street, and Second Apadana street, Isfahan, and three livestock and slaughterhouses in Borkhar County, Fasaran, a village in Baraan-e Shomali Rural District, in the Central District of Isfahan County, and Najafabad County, Isfahan Province, Iran, from August 2018 to April 2019. All specimens were divided into two parts: one portion used for direct microscopic examination with potassium hydroxide 20%, and another part subcultured on sabouraud glucose agar (Difco, Detroit, MI, USA) with

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chloramphenicol (0.04 g/L) and cycloheximide (0.5 g/L) for dermatophytes and sabouraud glucose agar (Difco, Detroit, MI, USA) with chloramphenicol (0.04 g/L) and without cycloheximide for nondermatophyte molds and incubated at 35°C for 3 weeks.

- The inclusion criteria: resistant lesions to antibacterial agents
- The exclusion criteria: antifungal consumption and bacterial growth on culture media.

Molecular identification

DNA was extracted using phenol/chloroform method.[7] ITS1-5.8SrDNA-ITS2 region was amplified for sequence analysis.[8] Briefly, polymerase chain reaction (PCR) mixture including 2.5 µL of 10 × reaction buffer, 0.4 mM dNTPs, 1.5 mM $MgCl_2$, 1.25 U of Taq polymerase, 30 pmol of both ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers, and 2 µL of extracted DNA were applied in a final volume of 25 µL. The PCR cycling conditions were an initial denaturation phase at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with a final extension phase at 72°C for 7 min. Seven microliter of PCR products was loaded on 1.5% agarose gel, and stained with 0.5 µg/mL ethidium bromide, then visualized by gel documentation system (UVITEC, UK) and photographed. PCR products were purified, and cycle sequencing reactions in forward direction were performed (Bioneer, South Korea). The sequencing products were analyzed with Chromas 2.6.6 (https://technelysium.com. au/wp/chromas/) and were evaluated using of NCBI BLAST searches against fungal sequences existing in DNA databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Clinical specimens were obtained from sheep (29.3%), cow (25.6%), cat (12.2%), camel (9.7%) (from Borkhar), calf (7.3%), goat (6.1%), horse (4.9%) (from Najafabad), and dog (4.9%). Thirteen mold strains out of 82 clinical samples (15.8%) were isolated and identified. Male-to-female sex ratio was 2/22, 7/14, 6/4, 1/7, 3/3, 4/1, 1/3, and 1/3, for sheep, cow, cat, camel, calf, goat, horse, and dog, respectively. Age range was 1–3 years, 2–4 years, 6 month–3 years, 2–5 years, 5–8 months, 2–3 years, 3–6 years, and 4 months–4 years for sheep, cow, cat, camel, calf, goat, horse, and dog, respectively. Lesions were located on the ear (35.4%), abdomen (21.9%), neck (20.7%), trunk (10.9%), tail (4.9%), foot (2.4%), eyelid (2.4%), and muzzle (1.2%). The ITS1-5.8SrDNA-ITS2 region was amplified, and PCR products [Figure 1] were sent for sequence reaction in forward direction. *Acremonium exuviarum* (n = 4; 30.7%) [Figure 2], *Sarocladium implicatum* (n = 2; 15.4%), *Arthroderma otae* (n = 2; 15.4%) [Figure 3], *Chaetomium iranianum* (n = 1; 7.7%), *Trichothecium roseum* (n = 1; 7.7%), *Lichtheimia ramosa* (n = 1; 7.7%) [Figure 2], *Penicillium chrysogenum* (n = 1; 7.7%), and *Microsporum equinum* (n = 1; 7.7%) [Figure 3] were isolated and identified from clinical specimens. Table 1 shows the characteristics of animals with lesions suspected to dermatomycosis in the present study.

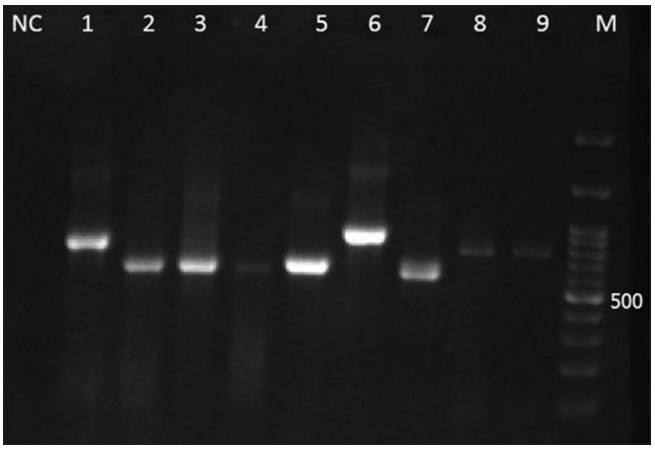

Figure 1: Agarose gel electrophoresis of polymerase chain reaction products. NC: Negative control, lane 1: *Arthroderma otae*, lane 2: *Acremonium exuviarum*, lane 3: *Sarocladium implicatum*, lane 4: *Chaetomium iranianum*, lane 5: *Trichothecium roseum*, lane 6: *L. ramose*, lane 7: *Penicillium chrysogenum*, lane 8: *Microsporum equinum*, lane 9: *Arthroderma otae*, and M: 100 bp DNA size marker

Discussion

Dermatomycoses have a worldwide distribution, with high frequency in the industrialized countries. Etiologic agents contain opportunistic fungi (*Aspergillus*, *Trichosporon*, *Rhodotorula*, *Acremonium, Scopulariopsis, Rhizopus*, *Candida, Cryptococcus*.) and dermatophyte species[9-11] In the present study, we isolated rare molds from various lesions in animals mimicking dermatophytosis. Three isolates belonged to dermatophyte genus including *A. otae* (2 isolates; obtained from cats), and *M. equinum* (1 isolate; obtained from a horse). Interestingly, all *A. exuviarum* strains were isolated from sheep. This uncommon mold isolated by Sigler *et al*.[12] from shed reptile skins for the first time and identified based on β-tubulin and ribosomal internal transcribed spacer sequences. *Acremonium* is a large fungal genus that contains almost 160 species, most of them are in soil and phytopathogens and others are considered as humans and animals opportunistic pathogens.[13] Infections in mammals generally caused by traumatic implantation of the mold into the eye and skin; however, the role of *Acremonium* genus as a causative agent of onychomycosis has also been reported.[14] *Sarocladium* genus was previously classified in the *Acremonium* complex, however, regarding recent molecular investigation, the taxonomy of *Acremonium* was altered and some important animal and phytopathogenic species transferred to *Sarocladium* as a separate genus.[15] *S. implicatum* (*A. implicatum*) has been isolated

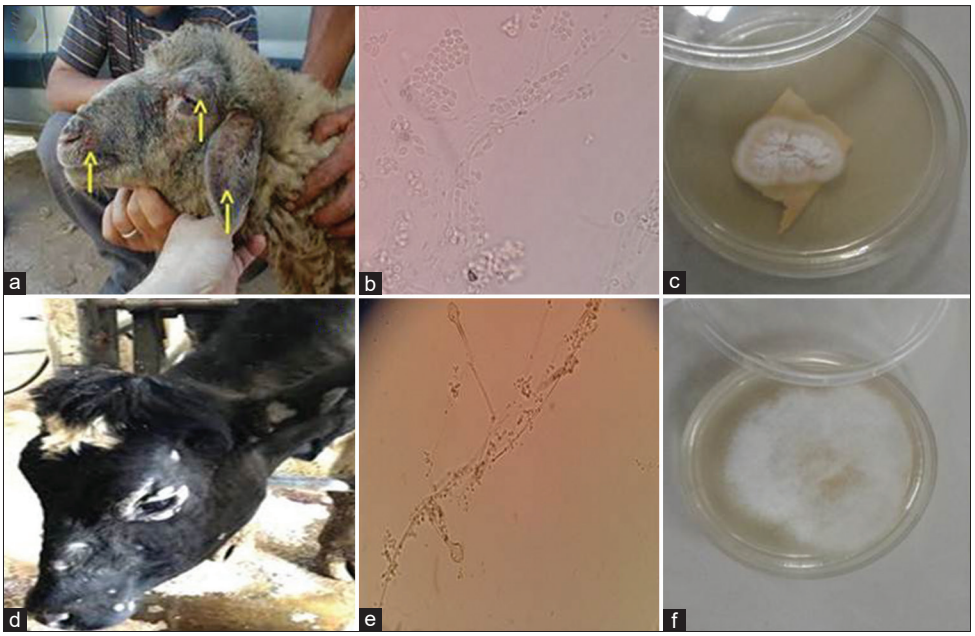

Figure 2: (a-c) clinical signs, microscopy, and culture of *Acremonium exuviarum* in a sheep, and (d-f) clinical signs, microscopy, and culture of *Lichtheimia ramosa* in a calf, in the present investigation

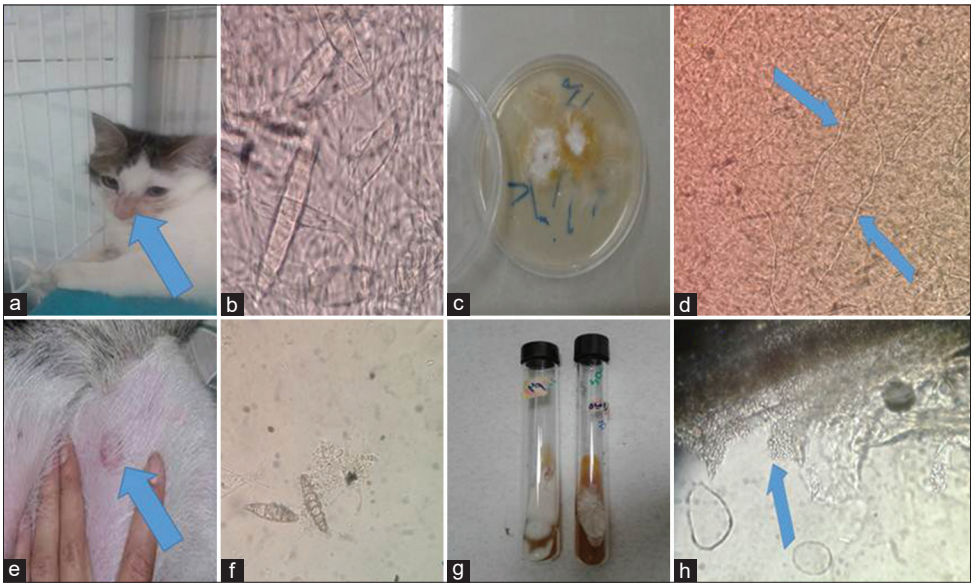

Figure 3: (a-d) clinical signs, microscopy, culture, and direct examination of *Arthroderma otae* in an infected cat, and (e-h) clinical signs, microscopy, culture, and direct examination of *Microsporum equinum* in an infected horse in the present study

from different clinical specimens such as sputum, bronch wash, sinus, bone, and bronchoalveolar lavage,[16] but we isolated this fungi from ear lesions in sheep and cow. *A. otae* complex comprises three species of *Microsporum*, including *M. canis* as a zoophilic species, the anthropophilic *M. ferrugineum,* and *M. audouinii* species.[17] *Microsporum canis* is a zoophilic species with worldwide distribution. Dogs, cats, and horses are natural reservoirs, and humans can be infected after contact with infected animal or human. We isolated two *M. canis* strains from muzzle and trunk of two cats referred to a specialized pet clinic. The genus *Chaetomium* is an olivaceous nondermatophytic mold found in plant debris, soil, and environment as opportunistic fungus. *Chaetomium* species are scarcely involved in human and animal infections, however, it can cause superficial (onychomycosis), subcutaneous, and disseminated infections in immunosuppressed patients.[18-20] *C. iranianum* is a member of the *C. carinthiacum* species group, characterized by hairs and fusiform ascospores and spirally coiled ascomatal hairs.[21] We obtained one isolate *C. iranianum* from ear lesion of a sheep in the present investigation. *T. roseum* is an ascomycetous fungus first reported in 1809, which produces different kinds of mycotoxins, such as trichothecenes and roseotoxins, which

Table 1: The characteristics of animals with lesions suspected to dermatomycosis in the present study

Number	Kind of animal	Sex	Age (year)	Location of lesion	Etiologic agent
1	Cat	Female	1	Muzzle	*A. otae*
2	Cat	Female	2	Trunk	*A. otae*
3	Sheep	Female	2	Neck	*A. exuviarum*
4	Sheep	Female	3	Ear	*A. exuviarum*
5	Sheep	Female	2	Ear	*A. exuviarum*
6	Sheep	Female	2	Ear	*S. implicatum*
7	Sheep	Female	1	Ear	*A. exuviarum*
8	Sheep	Female	2	Ear	*C. iranianum*
9	Cow	Female	3	Ear	*S. implicatum*
10	Cow	Female	2	Ear	*T. roseum*
11	Calf	Female	5 months	Neck	*L. ramosa*
12	Calf	Male	6 months	Ear	*P. chrysogenum*
13	Horse	Male	6	Neck	*M. equinum*

A. otae: Arthroderma otae, A. exuviarum: Acremonium exuviarum, S. implicatum: Sarocladium implicatum, C. iranianum: Chaetomium iranianum, T. roseum: Trichothecium roseum, L. ramose: Lichtheimia ramose, P. chrysogenum: Penicillium chrysogenum, M. equinum: Microsporum equinum

can spoil fruit crops.[22] So far, this mold has not been isolated from clinical samples of human or animals, thus isolation of this phytopathogen mold from ear lesion in the present study is questionable. Another rare isolated mold was *P. chrysogenum* obtained from a 6-month-old calf's ear lesion. Infection due to *Penicillium* species is rare; however, a number of superficial or systemic infections have been reported in human, such as otomycosis, onychomycosis, keratitis, alveolitis, esophagitis, endocarditis, and peritonitis.[23-28] Wigney *et al*.[29] reported an osteomyelitis associated with *P. verruculosum* in a German shepherd dog. *Lichtheimia* (*Absidia*) belongs to the order Mucorales containing six species, namely *L. corymbifera, L. ramosa, L. hyalospora, L. brasiliensis, L. sphaerocystis,* and *L. ornata*[30,31] Roden *et al*.[32] showed that *Lichtheimia* spp. accounted for nearly 5% of all mucormycosis in the USA, but it was identified as the second most prevalent causative agent of mucormycosis in Europe (19%–29%).[33] We isolated this fungus from neck lesion of a calf. The animal's wound had become chronic and was resistant to topical antifungal agents. *M. equinum* was another dermatophyte species caused a single lesion in the neck of a 6-year-old horse. The infection was treated using chlorhexidine as a common disinfectant agent and daily washing by ketoconazole shampoos after 8 weeks. Shokri and Khosravi[34] isolated 255 fungal cases from 1011 suspected animals to dermatomycoses. The most prevalent fungal infections were dermatophytosis (49.7%), Malassezia dermatitis (45.4%), candidiasis (2.5%), aspergillosis (2.2%), and zygomycosis (0.2%). In the present study, we isolated 3 out of 13 dermatophyte spp. (23%) from infected animals. Khosravi and Mahmoudi[35] reported *Microsporum canis* as the most frequent dermatophyte isolate from domestic animals in Iran between 1994 and 1998. We also isolated *A. otae* (*M. canis*) as the most common dermatophyte from two cats. Aghamirian and Ghiasian[36] identified *Trichophyton verrucosum* as the causative agent of dermatophytoses among infected cows, however, none of the cows in our study had dermatophytosis.

Conclusion

Zoophilic dermatophytes have public health implications and can transmit to humans by frequent contacts, so complete care must be considered when dealing with the infected animals, especially for immunosuppressed patients. Since opportunistic fungi are increasing as etiological agents of dermatomycoses, isolation of these molds from wounds can be a warning to veterinarians, and daily cleaning of wounds with a proper disinfectant is recommended for prevention of fungal colonization.

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Conflicts of interest

There are no conflicts of interest.

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